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New hosts for *Bartalinia* and *Chaetopyrena* in China

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Abstract—Two anamorphic species, *Bartalinia robillardoides* and *Chaetopyrena penicillata,* are reported on a new host in China. Both endophytic fungi were isolated from stems of *Ephedra intermedia* and are described and illustrated.

Key words—*Amphisphaeriaceae*, appendaged coelomycetes, *Didymellaceae*, endophytes, taxonomy

Introduction

In the course of investigating the endophytic fungi of *Ephedra intermedia* in Gansu province, China, healthy stems were surface-sterilized in 1% sodium hypochlorite for 1 minute and then rinsed three times in sterilized distilled water, and tissues were plated onto PDA to obtain pure cultures. Two coelomycetous fungi were isolated. Based on their cultural characteristics, morphological comparison, and analysis of the internal transcribed spacer region of ribosomal DNA (rDNA-ITS), the fungi were determined as two coelomycetous species on a new host in China. *Chaetopyrena penicillata* is reported for the first time from China.

Materials & methods

Isolates and morphology

The morphology of the strains was examined on OA: oatmeal agar (30 g oatmeal, 1000 mL distilled water), MEA: malt extract agar (15 g malt extract, 1 g peptone, 20 g

dextrose, 15 agar, 1000 mL distilled water) and PDA: potato dextrose agar (200 g potato, 20 g dextrose, 15 g agar, 1000 mL distilled water), incubated for 10 d at 25°C in the dark. All structures were described from 1-month-old PDA cultures.

Specimens are deposited in the Herbarium of Gansu University of Traditional Chinese Medicine, Lanzhou, China (HTCM).

DNA extraction and sequencing of (rDNA-ITS)

Mycelium was scraped from cultures growing on PDA, put into 100 mL PD, agitated using an orbital shaker set at 45 r.p.m. and incubated at room temperature under constant illumination by fluorescent lights. Mycelium (100 mg) was removed after 7 days and transferred to a mortar and pestle for grinding with liquid nitrogen. DNA extraction was then carried out using the FastDNAkit (SK1375, Sangon Biotech Co. Ltd.) following the manufacturer's instructions. The presence of DNA was verified by separation on a 0.8% agarose gel in 1×TAE (40 mM Tris-acetate, 1 mM EDTA, pH 8.0) stained with ethidium bromide (0.5 mg/L) and visualized under UV light. The ITS region was amplified using the universal primers, ITS1 and ITS4 (White et al. 1990). PCR was performed in 50 µL reaction volumes containing 10–100 ng of genomic DNA, 2.5 µL of each primer (10 µM), 0.25 µL dNTP (10 mM), 2.0 unit Supertaq DNA polymerase and 5 µL 10 × PCR buffer (Sangon Biotech Co. Ltd.). The amplification was performed in a thermocycler (Biometra Tg PCR). The PCR thermal cycle program was as follows: 95°C for 3 min, followed by 30 cycles of denaturation at 94°C for 3 min, annealing at 52°C for 45 s, and elongation at 72°C for 1 min, with a final extension step of 72°C for 8 min. The PCR products, spanning approximately 500 bp of ITS, were checked on 1% agarose electrophoresis gels stained with ethidium bromide. The PCR product was purified and sequenced using the above-mentioned primers by Sangon. Approximate phylogenetic placements were determined with the Blastn search option of the National Center for Biotechnology Information (NCBI) and the suggested identities are discussed in the descriptive notes below.

Taxonomy

Bartalinia robillardoides Tassi, Bull. Lab. Orto Bot. Reale Univ. Siena 3: 5. 1900.

Plate 1

Black conidiomata in PDA culture, stromatic, globose or subglobose, immersed or semi-immersed, solitary, glabrous, 605–757 × 585–702 µm diam, black to brownish black, wall thick, composed of bright brown, thick-walled cells, paler and thinner in inner layers. Ostiole absent, dehiscence irregular by breakdown of upper wall. Conidiophores absent. Conidiogenous cells cylindrical to subcylindrical, hyaline, 6–9 × 3 µm. Conidia cylindrical or fusiform, smooth-walled, straight to slightly curved, 4-septate, slightly constricted at septa, hyaline, 25.6–33.3 × 2.6–3.1 µm, length/width ratio 9.9. Apical cell hyaline, conic, thin-walled, 3.0 µm long, bearing (2–)3(–4) appendages from apex. Appendage branches 2–3, divergent, flexible, tapering gradually towards apex, 11.5–19.8 µm long. Median cells cylindrical, ochraceous

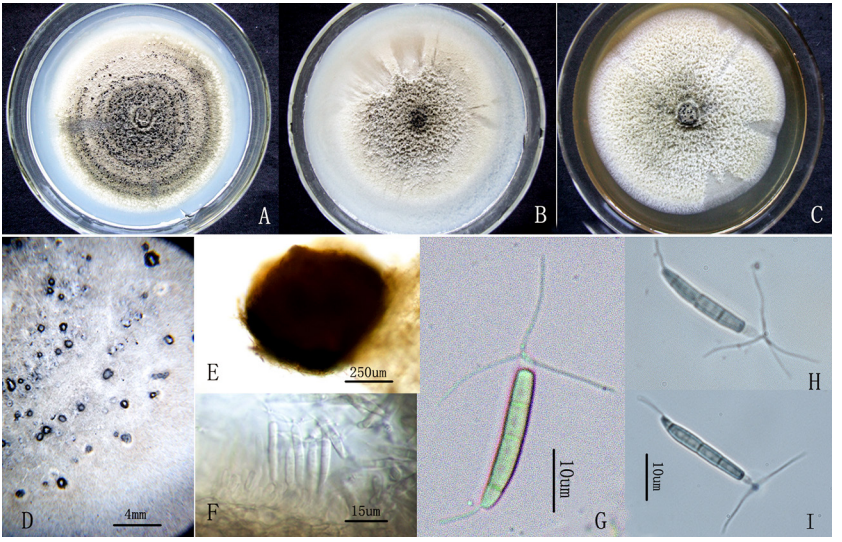

Plate 1. *Bartalinia robillardoides* (HTCM 1009024). A–C: Ten-day-old colonies on PDA (A), OA (B), and MEA (C); D, E: Conidiomata; F: Conidiogenous cells with developing conidia; G–I: Conidia.

to pale brown; the cell next to basal cell longest, 7.7–11.5(–14.1) µm (mean = 10.1 µm), l : w = 3. Basal appendage straight, (5.1–)7.7–9.5(–10.3) µm long (mean = 8.5 µm). Colony on PDA at 25°C grayish green, regular, with darker concentric zones, aerial mycelium floccose, slightly grey black in reverse, producing numerous black conidiomata, growth rate 7.3 mm/d. Colony on OA grayish green, no concentric zones, the reverse grey, producing sparse brown conidiomata, growth rate 6.5 mm/d. Colonies on MEA white to grayish white, sparse conidiomata, growth rate 6.3 mm/d.

Specimen examined: **China**: **Gansu Province**, Lanzhou city, Renshou mountain, isolated from stems of *Ephedra intermedia* Schrenk & C.A. Mey. (*Ephedraceae*), 20 Sep, 2009, Yan Wang. (HTCM 1009024; dried cultures, HTCM–F: 09122, 09123; GenBank, KF656706).

Comments — *Bartalinia robillardoides* is reported from diverse hosts around the world, including Hong Kong, China (Zhuang 2001, as *Seimatosporium robillardoides*), but it has not previously been reported from *Ephedra intermedia*. The identification of this isolate was made based on conidial morphology as well as on molecular sequence data. The Blastn search results of the ITS sequence of our Chinese isolate from *Ephedra* (GenBank KF656706) showed 99% similarity to *B. robillardoides* (GenBank EU552102), 100% similarity to

Ellurema sp. (GenBank AY148442), 96 and 95% similarity to two isolates of *B. pondoensis* (GenBank GU291796, AF405302), and 95% similarity to *B. laurina* (GenBank AF405302).

Nag Raj (1993), who published a comprehensive account of *Bartalinia,* accepted and keyed six species and listed another nine species as "unexamined and excluded taxa." Andrianova & Minter (2007) accepted and keyed ten species were by, and Marincowitz et al. (2010) added a futher species. Based on Nag Raj (1993), our *Ephedra* isolate keys to *B. robillardoides* based on its size and 4-septate conidia. Andrianova & Minter (2007) emphasized the importance of even or uneven conidial septation, conidial size, and number of appendage branches; in their key, our *Ephedra* isolate again keys to *B. robillardoides* because of the large cell next to the base, conidial size, and generally 3-branched apical appendage. The most recently described species, *B. pondoensis,* is unlike *B. robillardoides* in having 3-septate conidia. The genus *Ellurema* is applied to the sexual state of *Hyalotiopsis*, a genus that is distinguished from *Bartalinia* by characteristics of the conidial appendages (Nag Raj 1993).

Chaetopyrena penicillata (Fuckel) Höhn., Hedwigia 60: 132, 1918. Plate 2

Conidiomata superficial. Pycnidia single, solitary, globose to subglobose, dark brown to black, ostiolate, 320–400 µm diam, surrounded by setae, neck 100 × 50 µm; setae brown, thick-walled, septate, unbranched, gradually tapering upward, up to 150–180 µm long; wall consisting of 3–4 layers of brown textura angularis. Conidiophores hyaline, simple. Conidia hyaline, smooth, 1–celled, cylindrical to sub-cylindrical, straight, with rounded apex and truncate base, 12.6–17.7 × 2.5–5.1 µm (mean = 15.9 × 3.9 µm). Colonies on PDA 42 mm diam. after 7 days at 25°C in dark, smooth, black, margins grayish white, regular, reverse black, aerial mycelium sparse. Colonies on OA 36 mm diam after 7 days at 25°C in dark, substrate mycelium orange-green. Colonies on MEA reaching 30 mm diam. after 7 days at 25°C in dark; aerial mycelium grayish white; pycnidia not formed; chlamydospores not seen.

Specimen examined: **China**: **Gansu Province**, Lanzhou city, Renshou mountain, isolated from stems of *Ephedra intermedia* , 1 Oct. 2009, Yan Wang. (HTCM 1009028; dried cultures, HTCM–F: 09124, 09125; GenBank, KC492443).

Comments — A Blastn search of the ITS sequence of our Chinese isolate from *Ephedra* (GenBank KC492443) showed 99% similarity to *Chaetopyrena penicillata* (GenBank JQ663990). Our isolate agrees morphologically with this species as described in Arzanlou & Khodaei (2012). This genus is currently placed in the *Didymellaceae* (Gruyter et al. 2010). Although 19 species have been placed in *Chaetopyrena*, most species are obscure and do not have living cultures. Arzanlou & Khodaei (2012) reported *C. penicillata* as the cause of

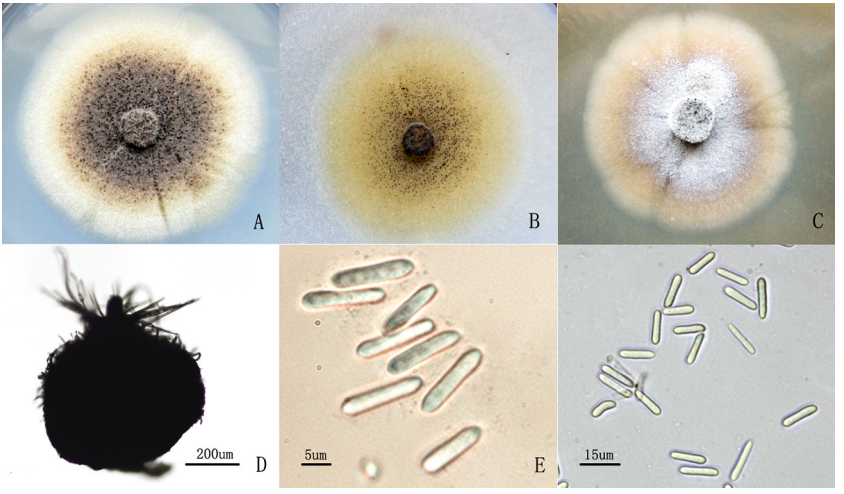

Plate 2. *Chaetopyrena penicillata* (HTCM 1009028). A–C: Ten-day-old colonies on PDA (A), OA (B), and MEA (C); D: Conidiomata; E, F: Conidia.

dry rot on Russian olive (*Elaeagnus angustifolia*). It is also reported from soil as well as dead twigs, fruit, and stubble in the Middle East, Russia, and South Africa. This is the first report of *C. penicillata* from China, and the first report from *Ephedra*.

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